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CLINICAL REVIEW

Why the dim light melatonin onset (DLMO) should be measured before treatment of patients with circadian rhythm sleep disorders

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SUMMARY

Treatment of circadian rhythm sleep disorders (CRSD) may include light therapy, chronotherapy and melatonin. Exogenous melatonin is increasingly being used in patients with insomnia or CRSD. Although pharmacopoeias and the European food safety authority (EFSA) recommend administering melatonin 1–2 h before desired bedtime, several studies have shown that melatonin is not always effective if administered according to that recommendation. Crucial for optimal treatment of CRSD, melatonin and other treatments should be administered at a time related to individual circadian timing (typically assessed using the dim light melatonin onset (DLMO)). If not administered according to the individual patient's circadian timing, melatonin and other treatments may not only be ineffective, they may even result in contrary effects.

Endogenous melatonin levels can be measured reliably in saliva collected at the patient's home. A clinically reliably DLMO can be calculated using a fixed threshold. Diary and polysomnographic sleep-onset time do not reliably predict DLMO or circadian timing in patients with CRSD.

Knowing the patient's individual circadian timing by assessing DLMO can improve diagnosis and treatment of CRSD with melatonin as well as other therapies such as light or chronotherapy, and optimizing treatment timing will shorten the time required to achieve results.

Sleep and circadian timing

Sleep is defined as a natural state characterized by a reduction in voluntary motor activity, a decreased response to stimulation and a stereotypic posture [1]. Despite the immense amount of sleep research, some of the function(s) of sleep remain elusive. To better understand how humans achieve consolidated sleep (and wakefulness), a two-process model of human sleep regulation has been proposed [2,3]. This model includes a homeostatic sleep drive (process S) determined by recent sleep–wake history, such that the longer you are awake the more homeostatic pressure to sleep is present [4]. The second element of the two-process model is the circadian rhythm (process C), which is largely independent of recent sleep and waking activity. The circadian process influences the timing, duration, and internal structure of sleep [5] and is regulated by a central endogenous circadian pacemaker (ECP) located in the suprachiasmatic nuclei (SCN) of the hypothalamus [6,7]. The ECP has a near-24-h period [8] which is synchronized to geophysical time by regular exposure to light and darkness [9].

Melatonin is a hormone produced by the pineal gland, and the timing of its secretion is strongly regulated by the ECP. In typical individuals keeping a regular sleep–wake schedule, melatonin levels are low to absent during the daytime, begin to rise in the evening, and peak during the nighttime hours. Melatonin secretion is suppressed by light [10], and therefore melatonin levels must be measured in dim light conditions to accurately reflect actual secretion. Serial sampling of melatonin measured in the blood or saliva can be used to assess circadian timing by determining the dim light melatonin onset (DLMO), the time at which levels rise above baseline [11]. Thus, measuring DLMO is a valuable tool in assessing circadian rhythm sleep disorders (CRSD).

Diagnosing circadian rhythm sleep disorders

CRSD are a group of sleep–wake disorders in which patients have problems with the timing of sleep due to a misalignment

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Abbreviations

ASPD	advanced sleep phase disorder
CBT	core body temperature
CRSD	circadian rhythm sleep disorders
DSPD	delayed sleep phase disorder
DLMO	dim light melatonin onset
ECP	endogenous circadian pacemaker
EFSA	European food safety authority
FDA	food and drug administration
ICSD	international classification of sleep disorders
LC-MS/MS	liquid chromatography tandem mass spectrometry
LOQ	limit of quantification
NOS	not otherwise specified
OTC	over-the-counter
PSG	polysomnography
RIA	radioimmunoassay
SCN	suprachiasmatic nuclei

between the timing of the internal biological clock and the timing of the desired sleep episode [12]. To state this in a simplified way, the patient cannot sleep when sleep is expected or needed. Currently seven distinct CRSD are recognized in the international classification of sleep disorders (ICSD) [13]. These are 1) time zone change (jet lag) syndrome, 2) shift work sleep disorder, 3) irregular sleep–wake pattern, 4) delayed sleep-phase disorder (DSPD), 5) advanced sleep-phase disorder (ASPD), 6) non-24-h sleep–wake disorder and 7) circadian rhythm sleep disorder not otherwise specified (NOS). Sack et al. extensively reviewed these CRSD in 2007 [14,15]. Several types of assessments are typically recommended to diagnose CRSD, i.e., sleep logs or diaries, questionnaires, actigraphy, polysomnography, and/or circadian phase (timing) markers. The 24-h endogenous melatonin rhythm is a rhythm driven by the central circadian pacemaker, and the timing of the onset of melatonin secretion in the evening is strongly associated with the timing of sleep in normal individuals [14–16]. Therefore, DLMO is a useful marker of the timing of the 24-h melatonin rhythm [17] and thus a very useful diagnostic tool for diagnosing CRSD. DLMO is a tool that can be used to determine whether abnormal sleep timing is associated with abnormal circadian rhythm timing (and thus DLMO can confirm the presence of a CRSD). In DSPD, sleep is delayed while in the ASPD sleep timing is advanced. DLMO can be used to confirm DSPD and ASPD, as the melatonin rhythm is delayed or advanced by a similar amount as the sleep timing. In patients with an irregular sleep–wake pattern, DLMO will often occur at irregular times. In shift work and jetlag, DLMO would be expected to occur not when the patient is attempting to sleep, but at a different time, leading to difficulty initiating or maintaining sleep [14,15]. DLMO is also a potentially valuable tool in the differential diagnosis of several sleep disorders that are typically thought to have a non-circadian origin e.g., psychophysiologial insomnia, bad sleep hygiene, narcolepsy, idiopathic hypersomnia, obstructive sleep apnoea, depression related insomnia [12,18–21]. Clinical symptoms of these non-circadian disorders can sometimes mimic CRSD symptoms.

Rahman and colleagues determined that DLMO has a measureable added value in diagnosing CRSD such as DSPD [22]. When DLMO was combined with polysomnography, the added diagnostic value was 32.5%. They found that the clinical sensitivity and specificity of the DLMO in DSPD patients was 90.3% and 84.0%, respectively, as determined in a clinical setting. One important finding in the study by Rahman et al. was that in a number of patients with apparent DSPD (those documented to have late bed and wake times), there was no correspondingly late DLMO. This indicates that the late sleep timing in those patients was not caused by late circadian rhythm timing, and therefore typical treatments for DSPD (see below) would be unlikely to work. In non-clinical settings, at-home saliva collection for determining DLMO has been validated [23,24]. Table 1 summarizes the relevant scientific papers that support the use of DLMO for diagnosis of CRSDs.

Although assessment of circadian rhythm timing using the DLMO is in widespread use in research settings, and has been adopted in clinical settings in some countries (see below), currently there is no food and drug administration (FDA)-approved method for assay, nor standards for determining normality of DLMO timing. This has limited the use of the DLMO in assessing patients in the US.

Table 1
Scientific studies that support using DLMO in the optimal diagnosis and treatment of CRSD.

Authors, reference number	Topic of paper
Rahman SA et al. 2009 [22]	Clinical significance for DLMO measurement
Pullman RE et al. 2012 [24]	At home saliva collection validation of DLMO testing
Keijzer H et al. 2011 [23]	At home saliva collection success rate in measuring DLMO
Keijzer H et al. 2011 [23]	Reliability of DLMO determination with a five
Molina TA and Burgess HJ 2011 [68]	point partial saliva curve collected at home every hour
Lewy AJ et al. 1992 [30]	Timing of exogenous melatonin intake is crucial
Skene DJ et al. 1996 [90]	for optimal phase shifting
Mundey K et al. 2005 [31]	
Burgess HJ et al. 2008 [29]	
Dodson ER and Zee PC 2010 [27]	
Braam W et al. 2009 [41]	Meta-analysis concluding that melatonin timing
Van Geijlswijk IM et al. 2010 [42]	is necessary for optimal treatment success.
Buscemi N et al. 2006 [43]	Meta-analysis concluding that melatonin intake 1–2 h before desired bedtime did not significantly improve sleep
Burgess HJ et al. 2010 [35]	Dose dependent timing of melatonin intake
Van Geijlswijk IM et al. 2010 [36]	
Keijzer H et al. 2011 [23]	DLMO predicted from sleep logs not reliable
Wright H et al. 2006 [64]	in patients with possible sleep–wake disorders

DLMO: dim light melatonin onset; CRSD: circadian rhythm sleep disorders.

Treatment of circadian rhythm sleep disorders

CRSD can be treated with measures such as improving sleep hygiene, strengthening time-cues, chronotherapy, bright light, or melatonin as a chronobiotic drug [25], based on three pillars: 1) strengthening time clues (zeitgebers), 2) adequately timed bright light and 3) adequately timed exogenous melatonin [26,27]. The goal of all treatments for CRSD is to shift the timing of the circadian system so that the patient can sleep at a more appropriate time of day. Thus, for patients with DSPD, the goal of treatment is to shift circadian timing earlier, so that the patient can sleep at more conventional (earlier) times. Similarly, for patients with ASPD the goal of treatment is to shift circadian timing and sleep later. Adding to the complexity of treating CRSD, treatments such as bright light or melatonin have phase-dependent effects. That is, the same treatment administered at different times of day can produce completely different results, potentially worsening the underlying CRSD rather than improving it. For example, exogenous melatonin administered 5 h before DLMO maximally phase advances (shifts to an earlier hour) the melatonin rhythm and its associated sleep–wake rhythm. If melatonin is instead given 10 h after DLMO, the rhythms are delayed (shifted later) maximally [28–31]. Therefore,

knowing DLMO is not only important for a reliable diagnosis but is critical for a successful treatment outcome. Nevertheless, several pharmacopoeias and the European food safety authority (EFSA) recommend administering melatonin 1–2 h before desired bedtime without taking into account information about the patient's circadian phase timing [32–34].

The time at which exogenous melatonin should be administrated to shift circadian timing is also dependent on the given dose [35,36]. When the timing of circadian phase is unknown, there is an increased risk that, especially with high doses, exogenous melatonin will exert its effects over such a prolonged duration that circadian timing will be shifted in the wrong direction, or that shifts in the correct direction will be cancelled out by subsequent shifts in the opposite direction [37]. This has been reported in mentally disabled persons who were treated with melatonin with initial success, but when treatment was continued for 3–4 wk the initial sleep disturbance reappeared [38]. In this case, melatonin was initially timed accordingly to DLMO, yet "spill-over" occurred, which was attributed to slow metabolism of melatonin. Spill-over is a phenomenon that was first hypothesized by Lewy and Sack [39]. In another report, a direct relationship between low endogenous melatonin levels and slow melatonin metabolization was observed in persons with an autism spectrum disorder [40]. Thus, attention must be paid to both the timing and when melatonin treatment is indicated.

The importance of first assessing DLMO for melatonin treatment success was shown in two meta-analyses that included studies where exogenous melatonin was administered at a time relative to DLMO [41,42]. Both reported a significant decrease in sleep-onset latency. This is in contrast to a different meta-analysis in which the majority of studies administered exogenous melatonin that was not timed relative to DLMO, but given at a time relative to bedtime. In that case, no significant improvement of sleep was found [43].

Several studies conducted with children also suggest that the DLMO timing at which melatonin is administered can predict the success of that melatonin treatment. Van der Heijden et al. found that the efficacy of melatonin treatment was significantly related to pre-treatment DLMO [44], indicating that more delayed pre-treatment DLMO were associated with stronger advances of sleep onset after melatonin treatment. These effects were also seen in a placebo-controlled study in children who had both attention deficit/hyperactivity disorder and sleep onset insomnia, and in a dose finding study in children with chronic sleep onset insomnia [36,45].

Melatonin assay

Analytical techniques for the detection of melatonin levels in biospecimen are becoming more sophisticated. Limit of quantification (LOQ, or the lowest level of melatonin that can be measured with accuracy) is especially important to determine the phase of the endogenous circadian pacemaker using melatonin. Bioassays, radioimmunoassay (RIA) and gas chromatography mass-spectrometry were among the first techniques that could measure melatonin with a low LOQ using blood or plasma [46–50]. Measuring melatonin in saliva using an RIA is less invasive than using blood, and can also be quite accurate [51,52], although the level of melatonin present in saliva is only about one-third of that in plasma, and thus the LOQ for saliva assays are even more critical than for blood. Salivary melatonin assessment for determining DLMO is routinely used in some clinics with more than 1500 sleep disorders patients annually [23]. The disadvantage of using the currently-available salivary RIA is the high cost of reagents (monoclonal antibodies) and the labour required to perform the assay. An accurate determination of DLMO requires at least five sequentially collected saliva samples, and the current price is approximately €20 per sample (€100 total).

In recent years, liquid chromatography tandem mass spectrometry (LC-MS/MS) has been increasingly used to determinate melatonin and its metabolites [53–56], and melatonin quantification in saliva is possible with LC-MS/MS [57]. Although the investment cost of LC-MS/MS equipment is quite high, reagent costs are considerably lower compared with RIA. LC-MS/MS could be the future "gold standard" for measuring melatonin, especially in high volume laboratories and clinics. Ideally, a point-of-care testing device for measuring melatonin will be available in the near future. However, the challenge for both point-of-care testing and LC-MS/MS will be to get the limit of quantification (LOQ) as low as possible (<0.5 pg/mL).

DLMO determination

Under normal conditions melatonin concentration increases during the evening, levels continue to increase during the sleep period, and begin to decline after mid-sleep [58]. During the day, melatonin is not produced in measurable quantities. However, in patients with CRSD or those with certain syndromes such as Smith-Magenis, the typical pattern of melatonin secretion may be altered or even inverted [59,60]. A typical melatonin curve is displayed in Fig. 1.

In healthy young individuals who have been maintaining a stable sleep–wake schedule with sleep at night, DLMO can be estimated with reasonable accuracy using sleep diaries, as their DLMO correlates with sleep onset time [61–63]. However, estimating DLMO based on sleep timing (using either a sleep log or polysomnography (PSG)) is far less accurate in individuals with irregular sleep–wake schedules, those who have travelled across time zones recently, and individuals with sleep–wake problems [23,64].

Measuring the entire 24-h melatonin profile using a series of saliva or blood samples collected in dim light is not only labour- and time-intensive, it requires a specialized laboratory [65,66]. Because of this, physicians typically estimate DLMO based on the patient history [67]. However, as noted above, this approach can result in

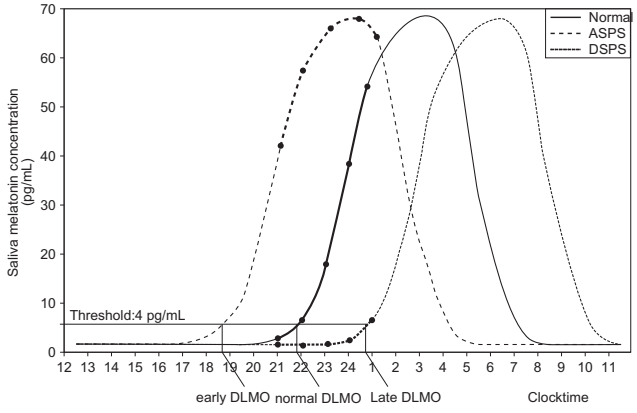

Fig. 1. Representative twenty-four pattern of melatonin secretion in a normal (solid line), ASPS (dashed line), and DSPS (dotted line) subject. Under typical conditions, melatonin levels are very low during the daytime, begin to rise in the evening just prior to usual sleep time, peak during mid-sleep, and fall back to baseline levels shortly after usual wake time. In our laboratory, DLMO is calculated at the point that the salivary melatonin concentration reaches the 4 pg/mL threshold. The round symbols in each curve indicate the five point sample collection, and those points are shown with a darkened line connecting them overlayed on the 24 h rhythm. When the partial five point curve is inconclusive (in the case of a low secretor, or when a patient has more extreme ASPS or DSPS) it is necessary to reassess the DLMO by changing starting time and/or extending the sampling window beyond five points, up to and including a full 24 h melatonin curve. ASPS: advanced sleep phase syndrome; DSPS: delayed sleep phase syndrome; DLMO: dim light melatonin onset.

either inaccurate diagnosis or inadequate treatment of CRSD [23,64].

A method to determine DLMO with greater reliability than sleep–wake history is to measure salivary melatonin levels for several hours around the time of the expected DLMO [17,23]. Critical for the success of this method is that the patient remains in dim lighting (<30 lux) for the entire sampling period, that they do not exercise, and that modest limits on certain foods and beverages are applied. Hourly saliva samples (in some cases collected using a Salivette®) are collected. This method produces a partial curve (e.g., typically five sequential points), which in most cases is sufficient to reliably determine DLMO. Collection of serial saliva samples for DLMO determination can be done in the home, and with sufficient instruction can produce reliable DLMO estimates in most individuals [23,24,68].

After assay, DLMO can be calculated from the serial samples by linear interpolation between adjacent points, looking for the time at which rising melatonin levels cross a pre-determined threshold. Lewy et al. recommended a fixed threshold of 10 pg/mL when melatonin is measured in serum [30]. Due to lower salivary levels [69], Nagtegaal et al. recommended a fixed threshold of 4 pg/mL in saliva [51], while others have suggested a 3 pg/mL threshold to determine DLMO from saliva [68,70]. In some patients, a fixed threshold to determine DLMO cannot be used, as their plasma or saliva levels never reach that threshold value. As an alternative, a relative threshold can be used to estimate DLMO in such "low secretors". One such relative threshold measure that has been suggested is to use the point at which levels rise more than two standard deviations above baseline levels [52]. However, this method requires that more samples (particularly baseline samples) be collected in order to have at least three baseline values to calculate the standard deviation.

Although multiple methods can be used to determine DLMO, there is no consensus about which method is best, and often a single method will not work best for all patients in a given group [17,71,70]. In a clinical setting, multiple methods to determine a DLMO are not necessary and a fixed threshold is the most convenient way of determining DLMO. However, previous studies suggest that a fixed threshold will work in only about three-quarters of patients. The remaining patients will consist of "low secretors" in whom alternative methods to calculate DLMO will need to be performed, and patients in which additional saliva sampling will be needed in order to determine DLMO [23], most likely due to an extreme delay or advance in the timing of their melatonin rhythm (Fig. 1). Using a standard starting time for collecting saliva samples in all patients results in missing the DLMO in patients who are extremely advanced or delayed. In such patients, additional saliva samples before or after the standard five point curve are necessary. In some patients it is necessary to determine a full 24-h profile because the partial profile does not provide sufficient information, such as in non-secretors or in individuals with reverse profiles.

In totally blind patients with a non-24-h sleep–wake disorder, where circadian rhythms are suspected to be free-running [72], it is necessary to perform multiple melatonin phase measurements at regular intervals to document the free-running status and to determine the period of the patient's circadian system [73,74]. Not all totally blind persons have free running rhythms [75], but in patients with a free running rhythm a correct diagnosis of the free-running status is required as they will require long-term treatment. Understanding the circadian period of the patient is also necessary in such patients in order to time the treatment appropriately [76]. In fact, this methodology was used in a recent application to the FDA for approval of a melatonin agonist to treat non-24-h sleep–wake disorder in blind patients [77].

The core body temperature (CBT) rhythm can also be used to determine circadian rhythm timing, but must be assessed using the highly specialized constant routine conditions, as CBT is strongly masked by changes in posture, activity level, and sleep–wake state [78]. Although CBT assessed in a constant routine protocol is routinely used in research settings, the amount of time (24+ hours), the strict postural and environmental control, and the invasive procedures required make determining circadian rhythm timing using CBT both impractical and too expensive for routine use in clinical settings [8,79].

Clinical experience

The Centre for Sleep-Wake Disturbances and Chronobiology of the Gelderse Vallei Hospital sees about 1500 patients annually with possible circadian-rhythm sleep–wake disturbances. In this section we present our clinical experience diagnosing and treating such patients.

Intake and preliminary investigation begins with an on-line questionnaire. If the response suggests a potential CRSD, a home test kit containing Salivettes® and instructions is sent to the patient to collect samples to assess DLMO [23]. The timing of the sample collection is determined by the age of the patient: in children aged 6–12 the hourly saliva samples are collected between 19:00h and 23:00h, in adolescents aged 13–16 y the samples are collected between 8:00h and 24:00h, and in patients older than 16 y the samples are collected between 21:00h and 1:00h Furthermore, the patient is asked to keep a one-week sleep diary on the internet. After receiving the results of the melatonin sampling and the sleep logs, the sleep physician discusses the results with the patient and may order further investigations (i.e., additional melatonin measurements, polysomnography, actigraphy, etc.), or starts treatment (strengthening time cues, improving sleep hygiene, light therapy, melatonin treatment). Usually the patient is asked to report the results of the treatment on the internet, a few days before he revisits the clinic.

In patients with delayed sleep phase disorder who are older than 16, we usually prescribe melatonin 5 h before DLMO (but not earlier than 19:00h), while in children aged 6–16 y we usually prescribe melatonin 2–3 h before DLMO (but not earlier than 18:00h). We start with a 1 mg dose of melatonin. If the patient reports no change in evening sleepiness or in morning alertness, the dose is increased by 1 mg every 1–2 wk until the patient feels a change in sleepiness at night or in the morning. The dose is then maintained. In patients younger than 10 y, the maximum dose we typically use is 3 mg, while in elderly patients the maximum dose we use is 6 mg. However, in isolated cases a higher dose is needed, in which case we do not exceed 10 mg. We do this stepwise increase in dose to prevent spill over, and use a 1–2 wk interval between dose increases because when the melatonin is adequately timed and dosed it shows an effect on reducing sleep onset latency within a few days [42,80]. Treatment efficacy is assessed using actigraphy or a sleep diary for 4–7 consecutive days and nights. In some patients, we combine bright light treatment with melatonin administration. For patients with advanced sleep phase disorder, the light treatment is done at or in the 2 h period after DLMO, while in patients with delayed sleep phase syndrome the bright light is administered in the morning.

At the sleep centre, an increasing number of patients are seen who were prescribed melatonin without knowing their DLMO. This treatment was ineffective, hence their referral to the sleep centre. Unfortunately, attempting to measure DLMO within a few weeks after stopping melatonin treatment is often inconclusive, resulting in re-testing a few months after stopping melatonin treatment. After re-testing, the majority of patients re-start treatment with melatonin timed to their individual DLMO, and this treatment is successful.

In our clinical experience, in some patients the effect of melatonin treatment decreases after a few weeks, although initial time of melatonin intake was optimal and there was no co-morbidity which might explain the decreasing effectiveness with time. In such patients, we typically measure salivary melatonin the day after intake of melatonin at the usual time in order to determine whether the DLMO timing has changed. We sometimes find that pre-DLMO (baseline) salivary melatonin levels are higher than typical, often >50 pg/mL (with typical levels <0.5 pg/mL). Melatonin is metabolized in the liver by cytochrome P450 enzymes to its primary metabolite 6-hydroxymelatonin, conjugated to sulphate, and excreted in urine [56,81,82]. Inhibitors, substrates, and inducers [83] can influence these cytochrome enzymes and subsequently influence melatonin concentration. We hypothesize that in certain patients, melatonin metabolizes more slowly than average, and we have evidence that in some cases this slow metabolism is due to expression problems of the *CYP1A2* gene [38]. This slow metabolism leads to continuously high levels of melatonin, and an associated reduction of treatment effect. In such patients, we lower the nightly melatonin dosage and can resume treatment success.

While treatment with melatonin in a subset of patients who are slow metabolizers can result in high levels of melatonin, melatonin treatment is relatively safe, side effects are limited [43,84–87], and toxicity is very low over a wide range of doses [88]. Despite concerns about the effect of long-term melatonin treatment on pubertal development, in our experience melatonin treatment in children can be conducted over a long period of time without substantial developmental side effects, although such treatment should be closely monitored [87,89]. However, more research is needed to understand the long-term effects of melatonin ingestion in different patient populations. A concern is that melatonin is increasingly recommended for patients with insomnia or used by such patients as an over-the-counter (OTC) self-care medicine. In Europe and the US it is readily available in doses up to 3 mg in drugstores and web shops. One issue associated with OTC availability of melatonin is that sleep physicians are frequently confronted with patients who have potential CRSD who have had unsuccessful treatment outcomes due to unsupervised and/or inaccurately timed usage of melatonin. As mentioned above, melatonin is quite safe but one issue that can arise with unsupervised use is the impact on the biological clock, especially adverse phase shifting. Therefore, we believe that the use of melatonin in patients with chronic sleep problems should occur only after their baseline circadian timing information is known, and should be supervised by a health care professional who is familiar with melatonin and its phase-dependent effects.

Conclusion

DLMO is a clinically relevant tool for the diagnosis and treatment of CRSD, and is used to discriminate CRSD from other non-circadian sleep disturbances. In the ideal situation, it should be assessed to determine the phase of the circadian rhythm before treatment commences to prevent adverse phase shifting effects and to speed treatment effects. While DLMO assessment is not the standard practice in most countries, there is accumulating evidence that DLMO measurement strongly benefits patient care by improving diagnostic accuracy and treatment efficacy. However, more research is needed to demonstrate the usefulness of DLMO measurements in clinical patient care, to optimize the DLMO testing protocol, to establish normal timing values, and to obtain regulatory approval in the US and other countries so that diagnostic use of the DLMO can be integrated into clinical practice.

Practice points

1) Measure circadian timing (DLMO) before treatment of CRSD.
2) Prescribe melatonin or light therapy relative to DLMO.
3) Sleep diaries are not a reliable estimate of circadian timing in patients with possible sleep–wake disorders.
4) Stop treatment when sleep–wake problems reappear.

Research agenda

1) Establish the clinical significance for measuring DLMO in a partial curve.
2) Determine the optimal DLMO testing protocol (number of samples, timing of sample collection).
3) Demonstrate that knowing DLMO will increase diagnostic accuracy.
4) Demonstrate that knowing DLMO will provide the best treatment outcome by shortening the time for symptoms to improve and/or improving symptoms in greater numbers of patients.
5) Develop new analytical techniques for measuring melatonin, in addition to current RIA methods.
6) Determine what definition of DLMO should be used in clinical practice.
7) Determine the duration that must elapse from prior melatonin use before an accurate DLMO can be measured.
8) Identify cause(s) for slow melatonin metabolization and/or biomarkers for individuals who are slow metabolizers.

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